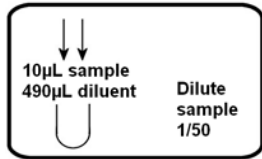
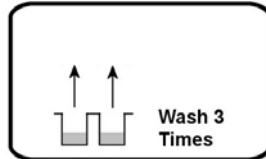


Test Procedure

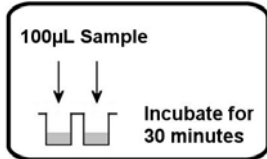
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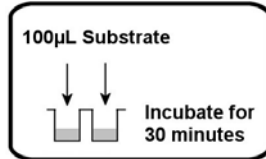
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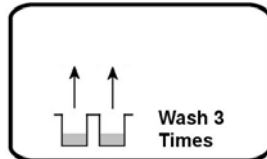
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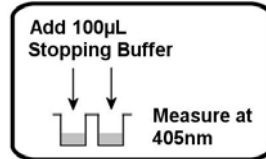
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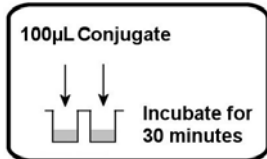
3.



7.



4.




AUTOZYME™ IFAB

Anti-Intrinsic Factor antibodies

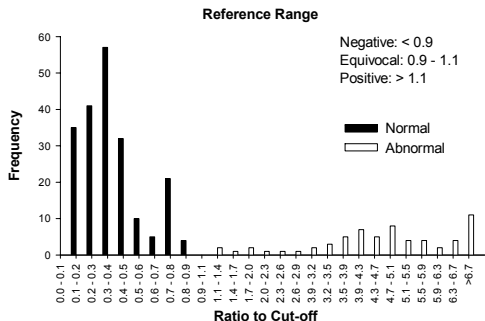
Code: Z4396

Instructions for Use

A5295.05
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13. Safety Precautions

For *in vitro* diagnostic use only.

For Professional Use only.

The **substrate** contains ABTS™ which is harmful if swallowed in copious amounts and may cause skin irritation if exposed for prolonged periods. In case of skin contact, wash with soap and water. Flush eyes with copious amounts of water.

The **calibrators and controls** contain human source material. Although found negative when tested for HIV-1 and HIV-2 antibodies, HCV and hepatitis B surface antigen, no test can guarantee their absence.

Therefore, the calibrators should be handled using the same safety precautions employed when handling any potentially infectious material.

Used calibrators, controls, samples, pipette tips and plates should be handled as clinical waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is recommended that gloves are worn when handling such items.

ABTS™ (2, 2'-azino-bis (3-ethylbenzothiazoline-6 sulphonic) acid) is a trademark of Roche Diagnostics.

Safety data sheets are available on request.

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Kit Contents Symbols

CAL	Calibrator (Cut-off Control)
CONTROL -	Negative Control
CONTROL +	Positive Control
BUF WASH	Wash Buffer
DIL SPE	Sample Diluent
CONJ	Conjugate solution
SUB	Substrate solution
STOP	Stop Solution
SORB	Solid Phase – Antigen Coated Wells

1. Intended Use

AUTOZYME™ IFAB Anti-Intrinsic Factor Antibodies Assay uses a peroxidase-ABTS system leading to the development of a green coloured reaction.

This assay allows the detection of antibodies to Intrinsic Factor (IF) in human serum.

2. Background

Biermer's anaemia is the disease most frequently associated with vitamin B12 deficiency.

This disease shows the classical features of megaloblastic anaemia (i.e. morphologic and functional abnormalities of the blood cells and marrow precursors related to impairment of DNA synthesis).

Pernicious anaemia is characterised by a gastric mucosal defect that decreases the synthesis of intrinsic factor. Since this glycoprotein plays an essential role in the transport and absorption of vitamin B12 across the small intestine, patients affected by pernicious anaemia show severe decreases in serum vitamin B12 levels.

The presence of auto-antibodies against gastric parietal cell cytoplasm, thyroid components and intrinsic factor, strongly suggest an auto-immune process to the disease.

Two types of anti-intrinsic factor auto-antibodies have been described. The blocking auto-antibodies (type I) hinder the binding of vitamin B12 complexes. Such antibodies are frequently encountered in patients affected by pernicious anaemia. Binding auto-antibodies (type II) that bind to the intrinsic factor as well to the IF/vitamin B12 complexes, and preventing their absorption, have also been described.

Detection of anti-intrinsic factor antibodies provides an important contribution to the differential diagnosis of pernicious anaemia (due to intrinsic factor deficiency) and other causes of vitamin B12 malabsorption. Indeed, other tests such as the cytomorphology of red blood cells, determination of serum vitamin B12 levels or the

The absorbances for the controls must be:

- > 0.8 for the positive control
- < 0.6 for the cut-off control
- < 0.4 for the negative control

The Ratio is expressed as a function of the cut-off value and is calculated for each sample as follows:

Ratio = sample absorbance / cut-off control absorbance

Interpretation

A sample is positive when the Ratio > 1.1

A sample is negative when the Ratio < 0.9

Samples giving a value between 0.9 and 1.1 should be regarded as equivocal.

11. Quality Control

Good laboratory practice requires that quality control samples be included in every run to check on assay performance. The kit control ranges are provided on the certificate of analysis.

If either control value falls outside the quoted range, the results are invalid and the assay should be repeated.

12. Clinical Sensitivity and Specificity

□ Positive population (n=63)

- Clinically affected patients with confirmed Biermer's anaemia and positive reference ELISA test for Anti-Intrinsic Factor antibodies.

■ Negative population (n=205)

- 205 normal blood donors.

Sensitivity of the test = 100%

Specificity of the test = 100%

4. Dispense 100 μL of each **kit control** or **diluted patient sample** into the appropriate wells. Incubate for 30 minutes at room temperature (18°C to 25°C). It is recommended that samples are tested in duplicate.
5. Gripping the frame on the long sides to retain the strips, flick out the contents of the wells. Using the diluted wash buffer, wash the wells three times either with an automated plate washer set to at least 300 μL per well, or by adding 300 μL to each well and flicking out, gripping the frame on the long sides to retain the strips. Alternatively use a wash bottle. Blot the wells on absorbent material to remove any residual liquid.
6. Add 100 μL **conjugate** to each well and incubate for 30 minutes at room temperature.
7. Gripping the frame on the long sides to retain the strips, flick out the contents of the wells.
Wash the wells three times using the same procedure as in step 5.
8. Dispense 100 μL **substrate** into each well, ensuring that it is initially pale green, and incubate for 30 minutes.
9. Stop the reaction by adding 100 μL of **stopping buffer**.
10. Measure the absorbance at 405nm on a 96 well microplate reader.

10. Calculation of Results

Three controls are included in the kit:

- a negative control representative of the normal population
- a cut-off control corresponding to the most elevated O.D. value obtained when a normal population (blood donors, n=110) was tested.
- a positive control containing anti-intrinsic factor antibodies.

Absorbance measurement: Semi-quantitative (Ratio) interpretation of results.

Read the absorbance at 405nm for each sample and control.

Schilling test are not specific enough for the diagnosis of a Biermer's anaemia.

Anti-Intrinsic Factor antibodies can be detected using two types of methodologies: RIA or ELISA methods. The ability of type I auto-antibodies to prevent the binding of vitamin B12 to the intrinsic factor has allowed the development of RIA methods.

Such methods, however, only detect type I auto-antibodies. Moreover, they are subject to interferences induced by exogenous sources of vitamin B12 that may induce false positive results (especially in the case of vitamin B12 treatment, or when the sample is taken after a Schilling test).

ELISA methods detect type I and type II antibodies and are unaffected by the presence of high endogenous vitamin B12 levels.

The AUTOZYME™ IFAB Anti-Intrinsic Factor Antibodies Assay is an easy, rapid and sensitive method allowing the detection of total anti-intrinsic factor antibodies. The use of purified intrinsic factor as antigen ensures the specificity of the method.

3. Principle

The AUTOZYME™ IFAB Anti-Intrinsic Factor Antibodies Assay is a solid phase (microwells) ELISA based on the 'sandwich' principle. The wells are coated with purified porcine intrinsic factor.

First Incubation

The diluted samples and controls are incubated in the microwells to form an intrinsic factor/anti-intrinsic factor complex. The unbound components are washed off.

Second Incubation

An anti-IgG coupled peroxidase conjugate is then added to the well and incubated. Any unbound conjugate is washed off.

Third Incubation

A very pale green substrate is then added to the wells. The intensity of the green colour formed is proportional to the amount of anti-intrinsic factor antibodies bound in the first incubation. The reaction is stopped with a low pH solution.

4. Kit Contents

1 vial sample diluent, 60mL (ready-to-use)
1 vial wash buffer concentrate, 100mL (x10)
1 vial conjugate, 15mL (ready-to-use)
1 vial positive control, 1.5mL (ready-to-use)
1 vial cut-off control, 1.5mL (ready-to-use)
1 vial negative control, 1.5mL (ready-to-use)
1 vial substrate, 15mL (ready-to-use)
1 vial stopping buffer, 15mL (ready-to-use)
1 foil sachet containing 1 set of intrinsic factor coated microwells
1 instruction leaflet
1 QC certificate

5. Storage

The kit should be stored refrigerated at 2-8°C. Do not use the reagents beyond their expiry date. Do not freeze. Keep all reagents away from direct sunlight.

6. Sample Handling

AUTOZYME™ IFAB may be performed on human serum or plasma samples. Use fresh or frozen samples within as short a time after collection as possible.

Collection

Serum - Collect 5mL of venous blood in an additive-free glass tube. Allow to clot at room temperature. Centrifuge, separate out the serum fraction and store at 2 - 8°C or in aliquots at -20°C for longer periods. Avoid repeated freezing and thawing.

Plasma - Collect 5mL of blood in a glass or plastic tube containing an anti-coagulant. Centrifuge, separate out the plasma and store at 2 - 8°C or in aliquots at -20°C for prolonged storage.

7. Additional Reagents and Equipment

Deionised or freshly distilled water.
Precision micropipettes to deliver 10-1000µL.
Multichannel micropipette or repeating dispenser to deliver 100µL.
100mL and 1000mL measuring cylinders for reagent preparation.

Automated plate washer (optional).
96-well microplate reader with 405nm filter.
Software package (optional).

8. Procedural Precautions

Numbering of each strip is advised prior to commencing the assay.

Allow all reagents to equilibrate to room temperature (18 - 25°C) before use for a minimum of two hours.

Avoid the use of icteric, lipaemic or grossly haemolysed samples.

Always change tips between different calibrators, samples or control sera to prevent sample carryover.

Never allow the same pipette tip to be used with different reagents. Special care is needed to prevent contamination of the substrate by the conjugate.

The substrate should be pale green. Any green colouration (absorbance >0.200) indicates substrate contamination and the substrate should be discarded. The well washing procedure is critical for the successful performance of the test, especially between conjugate and substrate incubations (i.e. the second and third incubations).

Do not use the kit beyond the expiry date given on the label. Unused reagents are stable at 2 – 8°C for up to 28 days after first opening the container. However, multiple re-use could increase the risk of reagent contamination. A reagent blank is recommended as a procedural control (100µL of sample diluent in duplicate).

9. Assay Procedure

1. Prepare the **wash buffer** as follows: dilute contents of the wash buffer concentrate (x10) vial to 1000 mL with deionised water.
2. Dilute patient samples 1/50 using the sample diluent e.g. 10 µL sample added to 490 µL diluent. The **kit controls** do not require dilution.
3. Remove the antigen-coated microwells from the resealable sachet. Reseal any unrequired wells in the resealable sachet, along with the desiccant sachet.